Synopsis

Social insects are the most dominant terrestrial fauna for the last 50 million years. This tremendous ecological success is accompanied by the fact that sociality has evolved multiple times independently and achieved highest degree of complexity in insect lineages. The remarkable social organization found in insect societies is the result of finely balanced cooperation and conflict among the colony members. A typical hymenopteran colony is characterised by one or a few queens monopolizing reproduction and several sterile workers cooperatively raising brood and performing colony activities. The colonies are often conceptualized as superorganisms where groups of cooperative workers are compared with organs in the body, each of which accomplish a particular task like brood care, foraging and defence. The choice of tasks is often regulated by a systematic age polyethism.

As the queens monopolize reproduction, they serve as the sole suppliers of eggs in the colony. Therefore, loss or death of the queen creates a crucial void

which exposes the colony to potential reproductive conflict for the position of egg-layer. This crisis is expected to be extreme in monogynous colonies. The situation is rescued only after a new queen is established, and the whole process is known as queen succession. I am interested in this crisis management, and my thesis deals with potential and realized conflicts associated with queen succession and behavioural strategies involved in resolution of these conflicts.

The queen can be replaced in two ways - either by a newly eclosed specialized reproductive individual, which happens in highly eusocial hymenopterans, or by an existing member of the colony (worker), as it happens in primitively eusocial hymenopterans. Unlike in highly eusocial species, the workers of primitively eusocial species retain their ancestral capability of mating and activating ovaries to produce both sons and daughters, which makes them suitable for taking up the role of queen in their lifetime. Hence, primitively eusocial species provide a unique situation where loss or death of the queen might result in severe reproductive conflict as the queen can be replaced by any one of the existing workers.

Strictly monogynous colonies of the tropical primitively eusocial wasp *Ropalidia marginata* provide ideal opportunities to study the reproductive conflict and its resolution associated with queen succession because the queen is fre-

quently replaced by one of her nestmates resulting in a serial polygyny. These queens have highly variable tenures of queenship ranging from seven to over 200 days, which, together with the fact that they are replaced by a variety of relatives such as daughters, niece and cousins, suggests a potential reproductive conflict with variable degrees of complexity.

I have divided my thesis in three parts which are as follows -

- **Natural queen turnover:** Previous works from this lab have tried to characterize the queen succession in *R. marginata* colonies by experimentally removing the queen from the colony. As this design involves the experimenter intervening at a random point of the colony cycle, the colony might not respond in the similar way as it might have done to a natural succession necessitated by loss or death of the queen. But rarity and unpredictability of natural queen turnovers made them difficult to study. Therefore, in this section, we gathered a dataset of long-term and opportunistic quantitative behavioural observations on eleven natural queen turnovers and compared them with available data on queen removal experiments. All our queen removal experiments resulted in a hyper-aggressive potential queen who gradually reduced her aggression, activated her ovaries and went on to become the unanimously accepted

new queen of the colony if the original queen was not returned. Here we found a similar phenomenon in natural colonies where a single unchallenged potential queen took over the colony as new queen after the old queen was lost, died or was driven out of the colony. In some of the natural colonies, the transition was preceded by aggression shown by the potential queens towards their nestmates including the queens, which indicates that they might have pre-empted the transition. The potential queens in natural colonies started laying eggs much faster than in experimental colonies suggesting their physiological readiness for the transition.

- **How does a colony respond to a declining queen?:** As we could show that some of the potential queens might perceive the upcoming queen turnover, a fair prediction would be that they sense it through the declining fertility status of the queens. Therefore, we tried to experimentally induce situations where the queen appears to be declining, expecting that it might lead to a queen turnover. The growing evidence suggests that *R. marginata* queen maintains her status by applying a pheromone on the nest surface by rubbing the tip of her abdomen. We knocked down the nest to deny the queen the surface for applying her pheromone, and argued that the queen would be overthrown as the

workers would sense her as infertile. To our surprise, the queen maintained her status in six out of seven colonies by applying her pheromone on the entire surface of the cage. However, the effectively insufficient concentration of pheromone elicited aggression from workers towards the queen, and the queen retaliated back with aggression. These results suggest that the pheromone, being an honest signal of fertility, is extremely important for the queen for maintaining her reproductive monopoly, and the workers are able to perceive the decline of the queen from her pheromone.

- **Queen-successor conflict over access to reproduction:** Here we more explicitly looked at the potential reproductive conflict between the queen and her successor over access to direct reproduction. We used the theory of parent-offspring conflict proposed by Robert Trivers (1974) as the conceptual framework and adapted it to unravel the pattern of queen-successor conflict in *R. marginata* colonies. According to this idea, we expected that there should be a pre-conflict zone where the queen and the successor both would agree that the queen should continue to reproduce, followed by a conflict zone where the successor would try to takeover but the queen would hang on, finally followed by post-conflict zone where they both would agree that the successor

should reproduce. To test this expectation, we maintained the queen and the potential queen on either side of a wire-mesh partition, each with randomly chosen half of the workers. It allowed the potential queen (successor) to establish herself and then we reintroduced the queen to her side of the mesh daily till the queen gave up. We could behaviourally characterise all three zones which always appeared in the expected sequence. The pre and post-conflict zones had no aggressive interaction between the queen and the potential queen, whereas the conflict zone was characterized by aggressive falling fight between them. This is our first success in experimentally creating overt conflict between the queen and her successor.

Overall we can say, that the queens and the potential queens of *R. marginata* show great behvioural plasticity which might have been shaped by natural selection as an adaptation for conflict resolution. We could show that the potential queens sometimes can predict the upcoming transition and prepare themselves accordingly, whereas they can also react to an experimentally created sudden loss of queen by hugely elevating their aggression. The docile queens, on the other hand, maintain their reproductive monopoly by a pheromone, which is essentially a feature of highly eusocial species. But these docile queens have not lost their capability to show aggression and

can use that to complement the insufficient concentration of her pheromone. This and the behaviour of potential queens in their establishment phase are strongly reminiscent of typical primitively eusocial species. We conclude that *Ropalidia marginata* is, perhaps, a particularly advanced primitively eusocial hymenopteran situated on an evolutionary continuum from primitive to highly eusocial species.